

CLAIMS

1. A nucleic acid construct comprising a chimeric promoter sequence and a cloning site for insertion of a coding sequence in operable linkage with the chimeric promoter, wherein the chimeric promoter sequence comprises:
 - (a) a hCMV immediate early promoter sequence;
 - (b) exon 1 and at least a part of exon 2 of the hCMV major immediate early gene; and
 - (c) a heterologous intron provided in place of the intron A region of the hCMV major immediate early gene.
2. A nucleic acid construct according to claim 1 wherein the hCMV immediate early promoter sequence (a) is SEQ ID No.1.
3. A nucleic acid construct according to claim 1 wherein exon sequence (b) is SEQ ID No.2.
4. A nucleic acid construct according to claim 1 wherein the heterologous intron (c) comprises a sequence selected from the group consisting of rat insulin gene intron A sequence, chicken keratin gene intron A sequence and chicken cardiac actin gene intron A sequence.
5. A nucleic acid construct according to claim 4 wherein the rat insulin gene intron A sequence comprises SEQ ID No. 3.
6. A nucleic acid construct according to claim 1 wherein the chimeric promoter sequence is SEQ ID No. 4.
7. A nucleic acid construct according to claim 1 which comprises a polyadenylation sequence.
8. A nucleic acid construct according to claim 7 wherein the polyadenylation sequence is derived from a polyadenylation sequence of a gene selected from the group consisting of rabbit β -globin gene, human papilloma virus (HPV) early or late genes, the HSV-2gB gene, a simian CMV immediate early gene and HSVgD late gene.

9. A nucleic acid construct according to claim 8 wherein the polyadenylation sequence is selected from the group consisting of SEQ ID NO:10, SEQ ID NO:11, SEQ ID NO:12 and SEQ ID NO:13.

10. A nucleic acid construct according to claim 1 which further comprises a signal peptide.

11. A nucleic acid construct according to claim 10 wherein the signal peptide is selected from the group consisting of human tissue plasminogen activator signal peptide (hTPA_{sp}), aprotinin signal peptide, tobacco extensin signal peptide and chicken lysozyme signal peptide.

12. A nucleic acid construct according to claim 1 wherein a coding sequence is provided in the cloning site.

13. A nucleic acid construct according to claim 12 wherein the coding sequence encodes an antigen.

14. A nucleic acid construct according to claim 13 wherein the antigen is an antigen of a viral, bacterial, parasitic or fungal pathogen.

15. A nucleic acid construct according to claim 14 wherein the antigen is HBsAg.

16. A nucleic acid construct according to claim 1 which is a plasmid vector.

17. A nucleic acid construct according to claim 1 wherein the nucleic acid is DNA.

18. A nucleic acid construct comprising:

(i) a chimeric promoter sequence which comprises:

- (a) a hCMV immediate early promoter sequence;
- (b) exon 1 and at least a part of exon 2 of the hCMV major immediate early gene; and
- (c) a heterologous intron provided in place of the intron A region of the hCMV major immediate early gene; and

(ii) a cloning site for insertion of a coding sequence in operable linkage with the chimeric promoter; and

- (iii) (a) a non-translated leader sequence which is derived from HBVpreS2 antigen sequence, HBV e-antigen sequence or HSV type 2gD antigen sequence and which is in operable linkage with the chimeric promoter; and/or
- (b) an enhancer sequence which is derived from a 3' untranslated region (UTR) of a HBsAg sequence, or a 3'UTR of a simian CMV immediate early gene sequence and which is in operable linkage with the chimeric promoter, wherein the enhancer sequence is downstream of the cloning site.

19. A nucleic acid construct according to claim 18 wherein the hCMV immediate early promoter sequence (a) is SEQ ID No.1.

20. A nucleic acid construct according to claim 18 wherein exon sequence (b) is SEQ ID No.2.

21. A nucleic acid construct according to claim 18 wherein the heterologous intron (c) comprises a sequence selected from the group consisting of rat insulin gene intron A sequence, chicken keratin gene intron A sequence and chicken cardiac actin gene intron A sequence.

22. A nucleic acid construct according to claim 21 wherein the rat insulin gene intron A sequence comprises SEQ ID No. 3.

23. A nucleic acid construct according to claim 18 wherein the chimeric promoter sequence is SEQ ID No. 4.

24. A nucleic acid construct according to claim 24 which comprises a polyadenylation sequence.

25. A nucleic acid construct according to claim 24 wherein the polyadenylation sequence is derived from a polyadenylation sequence of a gene selected from the group consisting of a rabbit β -globin gene, human papilloma virus (HPV) early or late genes, the HSV-2gB gene, a simian CMV immediate early gene and HSVgD late gene.

26. A nucleic acid construct according to claim 25 wherein the

polyadenylation sequence is selected from the group consisting of SEQ ID NO:10, SEQ ID NO:11, SEQ ID NO:12 and SEQ ID NO:13.

27. A nucleic acid construct according to claim 18 which further comprises a signal peptide.

28. A nucleic acid construct according to claim 27 wherein the signal peptide is selected from the group consisting of human tissue plasminogen activator signal peptide (hTPAsp), aprotinin signal peptide, tobacco extensin signal peptide and chicken lysozyme signal peptide.

29. A nucleic acid construct according to claim 18 wherein a coding sequence is provided in the cloning site.

30. A nucleic acid construct according to claim 29 wherein the coding sequence encodes an antigen.

31. A nucleic acid construct according to claim 30 wherein the antigen is an antigen of a viral, bacterial, parasitic or fungal pathogen.

32. A nucleic acid construct according to claim 31 wherein the antigen is HBsAg.

33. A nucleic acid construct according to claim 18 which is a plasmid vector.

34. A nucleic acid construct according to claim 33 which has the sequence in SEQ ID No. 14.

35. A nucleic acid construct according to claim 18 wherein the nucleic acid is DNA.

36. A nucleic acid construct according to claim 18 wherein the non-translated leader sequence is selected from the group consisting of SEQ ID No. 5, SEQ ID No. 6 and SEQ ID NO:7.

37. A nucleic acid construct according to claim 18 wherein the enhancer sequence is selected from the group consisting of SEQ ID No. 8 and SEQ ID No. 9.

38. A method of obtaining expression in mammalian cells of a polypeptide of interest, which method comprises transferring into said cells a nucleic acid construct comprising a chimeric promoter sequence

and a coding sequence encoding the polypeptide in operable linkage with the chimeric promoter, wherein the chimeric promoter sequence comprises:

- (a) a hCMV immediate early promoter sequence;
- (b) exon 1 and at least a part of exon 2 of the hCMV major immediate early gene; and
- (c) a heterologous intron provided in place of the intron A region of the hCMV major immediate early gene. .

39. A method according to claim 38 wherein the construct is delivered directly into a subject.

40. A method according to claim 39 wherein the construct is delivered by injection, transdermal particle delivery, inhalation, topically, orally, intranasally or transmucosally.

41. A method according to claim 40 wherein the construct is delivered by needleless injection.

42. A method according to claim 38 wherein the construct is delivered *ex vivo* into cells taken from a subject, and the cells are reintroduced into the subject.

43. A method according to claim 38 wherein the nucleic acid construct is coated onto carrier particles.

44. Coated particles, suitable for delivery from a particle-mediated delivery device, which particles comprise carrier particles coated with a nucleic acid construct wherein the construct comprises a chimeric promoter sequence and a coding sequence in operable linkage with the chimeric promoter, and wherein the chimeric promoter sequence comprises:

- (a) a hCMV immediate early promoter sequence;
- (b) exon 1 and at least a part of exon 2 of the hCMV major immediate early gene; and
- (c) a heterologous intron provided in place of the intron A region of the hCMV major immediate early gene.

45. Coated particles according to claim 44 wherein the carrier particles are gold or tungsten.

46. A dosage receptacle for a particle mediated delivery device comprising coated particles according to claim 44.

47. A particle mediated delivery device loaded with coated particles according to claim 44.

48. A particle mediated delivery device according to claim 47 which is a needleless syringe.

49. A method of nucleic acid immunisation comprising administering to a subject an effective amount of coated particles, which particles are suitable for delivery from a particle-mediated delivery device, the particles comprising carrier particles coated with a nucleic acid construct, wherein the construct comprises a chimeric promoter sequence and a coding sequence encoding an antigen in operable linkage with the chimeric promoter, and wherein the chimeric promoter sequence comprises

- (a) a hCMV immediate early promoter sequence;
- (b) exon 1 and at least a part of exon 2 of the hCMV major immediate early gene; and
- (c) a heterologous intron provided in place of the intron A region of the hCMV major immediate early gene.

50. A nucleic acid construct comprising:

- (i) a promoter sequence;
- (ii) a non-translated leader sequence derived from HBV preS2 antigen sequence, HBV e-antigen sequence or HSV type 2 gD antigen sequence; and
- (iii) a coding sequence operably linked to (i) and (ii)

wherein the coding sequence is heterologous to the non-translated leader sequence.

51. A nucleic acid construct according to claim 50 wherein the

promoter sequence (i) is selected from the group consisting of hCMV immediate early promoter sequence, Pseudorabies virus promoter sequence and Rous sarcoma virus promoter sequence.

52. A nucleic acid construct according to claim 50 wherein the coding sequence encodes an antigen.

53. A nucleic acid construct according to claim 52 wherein the antigen is an antigen of a viral, bacterial, parasitic or fungal pathogen.

54. A nucleic acid construct according to claim 50 which is a plasmid vector.

55. A method of obtaining expression in mammalian cells of a polypeptide of interest, which method comprises transferring into said cells a nucleic acid construct comprising:

- (i) a promoter sequence;
- (ii) a non-translated leader sequence derived from HBV preS2 antigen sequence, HBV e-antigen sequence or HSV type 2 gD antigen sequence; and
- (iii) a coding sequence encoding the polypeptide operably linked to (i) and (ii)

wherein the coding sequence is heterologous to the non-translated leader sequence.

56. A method according to claim 55 wherein the construct is delivered directly into a subject.

57. A method according to claim 56 wherein the construct is delivered by injection, transdermal particle delivery, inhalation, topically, orally, intranasally or transmucosally.

58. A method according to claim 57 wherein the construct is delivered by needleless injection.

59. A method according to claim 55 wherein the construct is delivered *ex vivo* into cells taken from a subject, and the cells are reintroduced into the subject.

60. A method according to claim 55 wherein the nucleic acid construct is coated onto carrier particles.

61. Coated particles, suitable for delivery from a particle-mediated delivery device, which particles comprise carrier particles coated with a nucleic acid construct comprising:

- (i) a promoter sequence;
- (ii) a non-translated leader sequence derived from HBV preS2 antigen sequence, HBV e-antigen sequence or HSV type 2 gD antigen sequence; and
- (iii) a coding sequence encoding a polypeptide of interest operably linked to (i) and (ii)

wherein the coding sequence is heterologous to the non-translated leader sequence.

62. Coated particles according to claim 61 wherein the carrier particles are gold or tungsten.

63. A dosage receptacle for a particle mediated delivery device comprising coated particles according to claim 61.

64. A particle mediated delivery device loaded with coated particles according to claim 61.

65. A particle mediated delivery device according to claim 64 which is a needleless syringe.

66. A method of nucleic acid immunisation comprising administering to a subject an effective amount of coated particles which are suitable for delivery from a particle mediated delivery device, the particles comprising carrier particles coated with a nucleic acid construct comprising

- (i) a promoter sequence;
- (ii) a non-translated leader sequence derived from HBV preS2 antigen sequence, HBV e-antigen sequence or HSV type 2gD antigen sequence; and

- (iii) a coding sequence encoding an antigen operably linked to (i) and (ii) wherein the coding sequence is heterologous to the non translated leader sequence.

67. A nucleic acid construct comprising:

- (i) a promoter sequence;
- (ii) a coding sequence operably linked to the promoter sequence (i); and
- (iii) an enhancer sequence 3' of and operably linked to the coding sequence (ii);

wherein the enhancer sequence (iii) is derived from a 3'UTR of an HBsAg sequence or a 3'UTR of a simian CMV immediate early gene sequence, and the coding sequence (ii) is heterologous to the 3' enhancer sequence.

68. A nucleic acid construct according to claim 67 wherein the promoter sequence (i) is selected from the group consisting of hCMV immediate early promoter sequence, Pseudorabies virus promoter sequence and Rous sarcoma virus promoter sequence.

69. A nucleic acid construct according to claim 67 wherein the coding sequence encodes an antigen.

70. A nucleic acid construct according to claim 69 wherein the antigen is an antigen of a viral, bacterial, parasitic or fungal pathogen.

71. A nucleic acid construct according to claim 67 which is a plasmid vector.

72. A method of obtaining expression in mammalian cells of a polypeptide of interest, which method comprises transferring into said cells a nucleic acid construct comprising:

- (i) a promoter sequence;
- (ii) a coding sequence encoding the polypeptide operably linked to the promoter sequence (i); and
- (iii) an enhancer sequence 3' of and operably linked to the coding sequence (ii);

wherein the enhancer sequence (iii) is derived from a 3'UTR of an HBsAg sequence or a 3'UTR of a simian CMV immediate early gene sequence and the coding sequence (ii) is heterologous to the 3' enhancer sequence.

73. A method according to claim 72 wherein the construct is delivered directly into a subject.

74. A method according to claim 73 wherein the construct is Delivered by injection, transdermal particle delivery, inhalation, topically, orally, intranasally or transmucosally.

75. A method according to claim 74 wherein the construct is delivered by needleless injection.

76. A method according to claim 72 wherein the construct is delivered *ex vivo* into cells taken from a subject, and the cells are reintroduced into the subject.

77. A method according to claim 72 wherein the nucleic acid construct is coated onto carrier particles.

78. Coated particles, suitable for delivery from a particle-mediated delivery device, which particles comprise carrier particles coated with a nucleic acid construct comprising:

- (i) a promoter sequence;
- (ii) a coding sequence encoding a polypeptide of interest operably linked to the promoter sequence (i); and
- (iii) an enhancer sequence 3' of and operably linked to the coding sequence (ii);

wherein the enhancer sequence (iii) is derived from a 3'UTR of an HBsAg sequence or a 3'UTR of a simian CMV immediate early gene sequence and the coding sequence (ii) is heterologous to the 3' enhancer sequence.

79. Coated particles according to claim 78 wherein the carrier particles are gold or tungsten.

80. A dosage receptacle for a particle mediated delivery device comprising coated particles according to claim 78.

81. A particle mediated delivery device loaded with coated particles according to claim 78.

82. A particle mediated delivery device according to claim 81 which is a needleless syringe.

83. A method of nucleic acid immunisation comprising administering to a subject an effective amount of coated particles which particles are suitable for delivery from a particle mediated delivery device, the particles comprising carrier particles coated with a nucleic acid construct comprising:

- (i) a promoter sequence;
- (ii) a coding sequence encoding an antigen operably linked to the promoter sequence (c); and
- (iii) an enhancer sequence 3' of and operably linked to the coding sequence (iii);

wherein the enhancer sequence (iii) is derived from a 3'UTR of an HBsAg sequence or a 3'UTR of a simian CMV immediate early gene sequence and the coding sequence (ii) is heterologous to the 3'enhancer sequence.

84. A purified isolated chimeric promoter sequence which comprises:

- (a) a hCMV immediate early promoter sequence;
- (b) exon 1 and at least a part of exon 2 of the hCMV major immediate early gene; and
- (c) a heterologous intron provided in place of the intron A region of the hCMV major immediate early gene